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The effect of anesthetics on renal tubular transport systems has not been previously studied free of the complicating factors of changing systemic blood flow, intra-renal flow distribution, levels of circulating hormones, and so forth. Previous work in this area of anesthetic pharmacology has been indirect, and has frequently been performed with preparations *in vivo*, where all of these complicating problems prevail. There have been many studies which utilize the transport mechanisms, assuming without proof that they function unaltered during anesthesia and surgery. Many of the clearance techniques for study of the kidney involve tubular secretion of some substance. This is illustrated by the following table:

Technique	Secreted Substance
Effective Renal Plasma Flow ($ERPF$)	Para-amino-hippurate (PAH)
Excretory Urography	Diodrast, Uroselectan, Topax Neo-iopax, Skiodan
Radio-isotope Renography	^{131}I -hippurate
Excretion Rate	Phenolsulfonphthalein (PSP)

(Pitts 1963)

All of these methods depend upon normal function of the "organic-acid active transport system" of the renal proximal tubular cell, which excretes the above substances and creatinine, chlorothiazide, probenecid, and a variety of glucuronides and sulfate esters. (Pitts 1963)

This system also excretes mersalyl, an acidic mercurial diuretic. The transport mechanism may not be directly involved with the diuretic action of the drug, for non-acidic congeners, such as chlormerodrin, have identical effects on sodium excretion without being themselves similarly transported. (Cafruny and Gussin 1967)

The system exhibits competition, reversible by mass action, between the various compounds transported. This phenomenon was recognised during the war years and capitalized on to conserve penicillin when it was so scarce. (Beyer et al. 1944; Pitts 1963) These competitive interactions can influence the function of diuretics of another chemical class. "Inhibition of furosemide-induced natruresis by probenecid suggests that adequate cellular concentration (and/or luminal concentration) rather than plasma concentration is a prerequisite to the natruretic action." (Hook and Williamson 1965) The dynamics of competition may even impinge upon the culinary artist, for excretion of the food dyes Amaranth (F.D. & C. Red No. 2), sunset yellow (F.D. & C. Yellow No. 6), and tartrazine (F.D. & C. Yellow No. 5) is through the same organic-acid system, and may be inhibited by probenecid or 4-amino-hippurate. (Despopoulos 1968)

This transport system is an active one, and is in consequence energy-dependent. (Pitts 1963) "Relatively low concentrations of azide, cyanide, and arsenite, as well as cold and anaerobiosis, block" the transport process. "All interfere with the oxidative processes which serve as sources

of energy to turn the secretory machinery. Dinitrophenol (DNP), which uncouples oxidation and phosphorylation, also depresses" the system. (Pitts 1963) DNP itself is actively transported by this same system, and its carriage may in turn be competitively inhibited by PAH. (Bernt and Grote 1968)

Another transport mechanism for "organic bases" also exists. Tetra-ethyl-ammonium and hexamethonium, potent ganglionic blocking agents, are excreted by it. The organic base secretory mechanism exhibits competitive phenomena. (Smith et al. 1967) Also, "morphine is transported by the base transport system of the kidney" and may be partially "metabolized by the renal tubular cells." (May et al. 1967) Yet another compound of interest to anesthesia secreted by this route is tolazoline, an α-adrenergic competitive blocker, used clinically prior to the introduction of phentolamine. (Goodman and Gilman 1965)

The two transport systems have similar mechanism, but are distinct in the compounds carried. They do not interact. Both show competitive effects within their respective chemical classes, and both appear to be in the proximal tubule. (Pitts 1963; Burg et al. 1966; Chambers and Kempton 1933) There are analogous secretory mechanisms in the liver (Despopoulos and Sorrenberg 1967) and in the ciliary body of the eye. (Sugiki et al. 1961) There may be others. Careful study has shown that "the cellular transport mechanism is fundamentally similar in the pro-, meso-, and metanephric kidneys." (Jaffee 1954)

SECRETORY MECHANISMS AND ANESTHESIA

Not long after its discovery, renal secretion was applied to the study of the pharmacology of diethyl ether. Miller and Cabot (1915) observed depression of phenolsulphonephthalein excretion, "Excretion Rate," with ether anesthesia and operation. The depression was increased by shock. Many subsequent studies have either used some measure dependent on renal tubular function or been directed at the secretory function itself.

Coller et al. (1943) used Diodrast clearance ($C_{Diodrast}$) in the study of effects of ether and cyclopropane on renal function in operated man with morphine and scopolamine as premedication, and found little change of $C_{Diodrast}$ with anesthesia, in the absence of major shifts in blood volume. Craig et al. (1945) found that their "deep" ether and cyclopropane anesthesia in dogs produced depression of urine flow, both glomerular filtration and renal blood flow (as measured by clearance techniques), and the maximal tubular transport of glucose, while "light" anesthesia had little effect. Burnett et al. (1949) reported a study in man, using mannitol clearance for glomerular filtration rate (GFR), and PAH for ERPF, comparing ether and cyclopropane before operation in unpremedicated patients in the first to second plane of the third stage of anesthesia, by clinical criteria. They noted substantial falls in ERPF, averaging 39% with ether and 52% with cyclopropane, in the absence of hypotensive episodes.

More recently, Habif et al. (1951) published observations on meperidine, cyclopropane, ether, and thiopental in man. They found substantial vasoconstriction of the renal vessels, as determined by clearance studies, and found that operation added little additional effect. Glauser and Selkurt (1952) studied barbiturates in dogs, finding that both pentobarbital and barbital reduced plasma flow in the kidney as measured by clearance techniques, 18% and 16% respectively. Miles and DeWardener (1952) reported that both ether and cyclopropane produced vasoconstriction in kidneys, using flowmeters rather than clearance methods. Miles et al. (1952) reported on the interaction of pentamethonium bromide with ether and cyclopropane. The depression of renal blood flow (measured by clearance techniques) by the two inhalants was not increased by the addition of the blocking agent. Hill et al. (1958) examined the renal effects of meperidine, phenobarbital, and chloral hydrate, and not only used low-level PAH excretion for ERPF, but also studied the maximal transport capabilities of the tubules (T_MPAH), showing awareness that there might be complex dynamics in renal tubular function.

There has been further expansion of the work in the past decade, and an increasing utilization of more sophisticated methods. Mazze et al. (1963) presented data on halothane, and in man, found with standard PAH clearance measurements that there was a dose-response pattern. C_{PAH} was 60% of control

for 0.5-1.0% halothane but was further decreased to 48% of control values for 1.2-3.0%. Their patients were premedicated, with morphine and scopolamine, and were operated upon. In a review of renal effects of drugs used in anesthesia, with presentation of some new data, Papper and Papper (1964) suggested that it may be essential to measure renal vein PAH in order to use the standard clearance techniques with reliability, as there may be changes in extraction due to drug effects. Miller et al. (1965) and Miller et al. (1966) pointed out that the original data on halothane had included the pathologic finding in dogs of proximal tubular dilatation (without necrosis and without histochemical alteration in alkaline phosphatase or non-specific esterase, Raventós 1956). They studied T_MPAH in detail, in unpremedicated patients without use of barbiturates or relaxants. The first study suggested some depression of tubular function by halothane, but the second study, taking account of changes in blood pressure, showed only changes proportional to the levels of hypotension induced, both by halothane and by halothane-nitrous oxide mixture.

Deutsch et al. (1966) also reported on the effects of halothane. They used ethanol infusions to overcome the antidiuretic state often associated with anesthesia, so that urine flow would be adequate for reliable clearance studies. Their patients were not premedicated and were studied prior to the initiation of operative manipulation. In addition,

renal vein PAH was determined in several, to assess the propriety of using PAH clearance for ERPF. With 1.5% halothane, they observed 38% decrease in renal blood flow. Deutsch et al. (1967) in similar experiments found that cyclopropane 19% (in end-expiratory air) produced a 42% depression in renal blood flow. Renal vein PAH was not measured in this study. Kennedy et al. (1969) reported that peridurals, using local anesthetics, reduced GFR in proportion to the accompanying decrease in blood pressure, but reduced ERPF even more. The effect was increased by the addition of epinephrine to the injection. They used ^{57}Co cyanocobalamin for GFR, following loading dose of cold B12, and ^{125}I-Hippuran for measuring ERPF.

As may be readily concluded, only the study by Deutsch et al. (1966) made the critical test of measuring renal vein PAH so that extraction could be ascertained. The data of Miller et al. from 1965 on transport maxima must be rejected for failure to control perfusion pressure, but the 1966 experiments by Miller et al. were suitably controlled. Most of the other studies have one or more practical or theoretical objections which invalidate them as detailed studies of renal tubular secretory function, suggesting that perhaps a more direct approach is necessary. This may be accomplished through examining the function of isolated tubules directly, thereby eliminating any question of indirect effects of the anesthetics by change of blood pressure, by change of intra-renal blood flow, by release of catecholamines, or by any other means.

ISOLATED TUBULE STUDIES

In the course of some basic research on tissue culture of chick kidneys, Chambers and Kempton (1933) observed the concentration of phenol red by cystic elements formed by the culture. They reported detailed studies of the phenomenon, stating that only cysts formed from proximal tubule of either the mesonephric 4½ day old embryo or the metanephric newly hatched chick would concentrate the dye in their lumina, and that the effect could be blocked by reducing the temperature to 3-6°C, suggesting some metabolic involvement.

Forster (1948) reported work with several types of kidneys, in thin slices, and also observed that fish kidneys could readily be separated into individual tubules, as they have less interstitial tissue than higher vertebrate kidneys. At that time, he described direct microscopic observation of these isolated tubules, analogous to the chick work, and gave some basic data on inhibition of phenol red transport by a variety of compounds. As further elucidated by Forster and Taggart (1950), the microscopic technique involved placing the isolated tubules into a bath of fish Ringers with a 1 mg% solution of phenol red. They graded intra-luminal concentration of the dye in the tubules and found that the time to first discernible concentration was 2-5 minutes in controls. The time to peak concentration was typically 30-60 minutes. Some tubules failed to function, and others performed poorly, presumably due to the trauma of disrupting the kidneys. The best functioning

units were used for grading. Many of the inhibiting chemicals mentioned earlier were first studied in this paper, including cyanide, azide, arsenite, phlorhizin, iodoacetate, fluoride, DNP, and mercury salts, as well as cold and anaerobiosis. They also described many of the competitive inhibitors, including PAH, penicillin, carinamide, and some antidiuretic derivatives of cinchoninic acid. Pack et al. (1952) added to the data on the effects of various ions, using the same experimental system, and found that the transport was apparently a two step process. Transport from the bath to the cell requires K^+ in the medium. and may be inhibited competitively with PAH, whereas the further transport from the cell to the lumen of the tubule requires the presence of Ca^{++} in the medium, and is not subject to competitive inhibition.

While studying the transport of a large series of closely related phenolsulfonphthalein dyes, Forster et al. (1954) observed in the same experimental arrangement that bromcresol green, the dye least efficiently transported, proved to be the most potent inhibitor of the others. They also noted that chlorophenol red "resulted in more uniform preparations than were obtained with phenol red. The bluish-red color of the former is more readily perceived and the stability of the color within the pH encountered in these preparations is a distinct advantage." Consequently, most subsequent work has used chlorphenol red in this experimental format.

The method was applied to the study of the two step nature of the transport by Forster and Copenhaver (1956) with adaptation to rabbit cortex slices. Forster and Hong (1958) and Hong and Forster (1958) again applied it, using flounder tubules. They added a "runout" technique, placing saturated tubules in medium free of dye, and observing subsequent changes. Hong and Forster (1959), again with flounders and the added runout technique, found that cell to lumen movement under these conditions could be influenced by Ca^{++}, phlorhizin, and competitive inhibitors. Forster and Goldstein (1961) reported a significant correlation of the activity of this transport system, as determined by T_MPAH, with renal succinoxidase activity, this enzyme being part of the Krebs cycle for aerobic metabolism. They studied a wide range of animals, including goosefish (Lophius sp.), frog, dog, cat, and rat. Burg et al. (1966) showed that the single tubule could be perfused under suitable microscopic controls, and demonstrated active PAH transport in proximal tubules of rabbits. Winter (1966) took the standard fish method to a high level of sophistication by arranging an adaptation to microspectrophotometry, and procured objective confirmation of earlier visual results.

Despite the reproducibility of the technique of using isolated fish tubules, certain anatomic questions remained. Concentration in the lumen implied that both ends of the open cylinder of an isolated tubule must spontaneously seal. The necessary proof of this, as well as data on the length of time an isolated tubule could remain morphologically unchanged, was

provided by Bulger and Trump(1965) and Trump and Bulger (1967). Their early study involved primarily the normal state of the sole, but their latter report showed many details of the transport phenomena. They found that fine structure, by light and electron microscopy, was maintained at least 6 hours in oxygenated balanced salt solution at 0-4°C, as was dye transport when the specimen was temporarily returned to 25°C. There was good correlation of capacity for dye transport with the maintenance of ultrastructure. Tubules showing damage showed no transport. Autophagocytosis was the principle injury reaction.

Several detailed reviews of renal transport mechanisms are of interest, summarizing the laboratory data and suggesting theoretical bases for understanding the phenomena, including Taggart (1958), Weiner and Nudge (1964), and Forster (1967). They describe methodology as well as results, primarily in terms of a carrier-mediated transport model.

EXPERIMENTAL METHODS

Using the methods of Forster and Taggart (1950) with chlorphenol red in lieu of phenol red (per Forster et al. 1954), adapted to the kidney tubules of goldfish (Carrassius autatus,L.), I studied the effects of thiopental sodium, nitrous oxide, halothane, and methozyflurane on the "organic acid" active transport system in isolated tubules in vitro.

The goldfish were purebred Shubunkin strain, obtained from the Stoutland Hatcheries, Stoutland, Missouri, as fingerlings. They were maintained in large tanks with aeration and filtration, and fed "Tetra Min " brand prepared large flake staple food, three weeks, alternating with fresh or fresh-frozen Tubifex worms, one week, until they were about one year of age. The tank water was always aged, de-chlorinated, de-fluorinated, buffered with "5-in-1 Water Conditioner" (by Aqua-Biotics, Inc.), and brought to 17-19°C before the fish were exposed to it. Feeding was at 9:00 A.M. daily and the tank room was in darkness from 5:00 P.M. until 8:30 A.M. daily. The laboratory itself was held at 18-20°C except for one day toward the end of the experiments when a power shut-down eliminated the air-conditioning and the temperature reached 22° in the tanks and 24°C in the laboratory.

The goldfish Ringer's solution had the following salts:

Compound	mMole/liter
NaCl	100.0
KCl	2.5
CaCl	1.5
$MgCl_2$	1.0
NaH_2PO_4	0.5
$NaHCO_3$	10.0

After removal, a pair of kidneys was placed in this solution and teased apart into separate tubules or clumps of tubules. These were stored in the same solution at $4^{\circ}C$ in shallow dishes. The experimental medium was Ringer's with chlorphenol red added to a concentration of 10^{-5} M plus the appropriate test substance dissolved in or perfusing the medium.

Studies were performed with the specimens in split Petri dishes (Falcon Plastics #1003 "Two Compartment Petri Dish" cut down to one-half its initial height to accomodate the short objective ditance of the 4X objective of a standard Nikon SBR microscope), either with the top removed, for the thiopental studies, or with the top on and cemented along the midline divider and edges with wedge-shaped cut-outs on either side for perfusing gas entry and exit. One side of the split Petri dish was always used for simultaneous control specimens. All studies were conducted under a ventilating hood at the measured room temperature, and the sub-stage lamp of the microscope was only turned on at the time of observations. A brief trial of controls confirmed the 6 hour or more true functional viability previously demonstrated by Trump and Bulger, and indeed one of these still had functioning tubules more than 24 hours after sacrifice, although the specimen had a very large amount of autolysis at that time microscopically. As U.S.P. Sodium Thiopental for Injection is highly alkaline, experiments run with it were done in the presence of 0.01 M secondary-tertiary sodium phosphate buffer mix, pH 7.4,

which itself was tested for effect. Experiments with nitrous oxide were performed with nitrogen as a control gas, both in oxygen. Halothane and methoxyflurane were vaporized with oxygen, and hence were tested against oxygen controls. Both the time to first discernible concentration and the time of peak concentration was obtained on each trial.

When using the gas or the vapors, the apparatus was set up an hour early and perfused to allow equilibration with the medium and saturation of the connecting tubing. Halothane was vaporized with a Fluotec Mark II and methoxyflurane was vaporized with a Foregger Copper Kettle in a 20°C water bath. Control of gas flows was with conventional rotameter-type gas flow meters and standard anesthesia equipment. Flows were kept high enough for reliable vaporization, with a bleed point added to the circuit just before the bubbler to vent excess gases to the hood.

Data for each series was compared to its simultaneously conducted control series, and means and sample standard deviations computed for each. The two-sample t test was the index of significance for difference between the means. (Freund 1962) Standard tables of t values came from the Handbook of Chemistry and Physics. (Weast et al. 1964)

EXPERIMENTAL RESULTS

TEST CONDITIONS	TIME TO FIRST DETECTABLE CONCENTRATION Control+	Test	n	P*
Buffer	7.1±1.27 min.	6.8±1.64 min.	9	NS
Buffer + 20 mg% Thiopental Na	8.4±1.99	22.6±3.51	7	S
Buffer + 10 mg% Thiopental Na	6.5±0.84	9.5±1.38	6	S
80% Nitrous Oxide	9.1±1.56	13.7±3.50	12	S
1.2% Methoxyflurane in O_2	5.9±1.46	8.4±2.32	8	NS
2.0% Halothane in O_2	6.3±1.75	7.0±1.79	6	NS
(Derived values comparing 20 mg% Thiopental Na with 10%)	20 mg% 22.6±3.51	10 mg% 9.5±1.38	7/6	S

*S = significant, $P \leq 0.05$; NS = not significant, $P > 0.05$
+ Mean ± Sample Standard Deviation

EXPERIMENTAL RESULTS, CONTINUED

TEST CONDITIONS	TIME TO MAXIMUM CONCENTRATION Control+	Test	n	P*
Buffer	27.9±4.36 min.	32.6±3.80 min.	9	NS
Buffer + 20 mg% Thiopental Na	35.3±3.16	59.1±5.48	7	S
Buffer + 10 mg% Thiopental Na	39.2±1.25	59.8±8.87	6	S
80% Nitrous Oxide	37.1±3.46	43.7±8.45	12	NS
1.2% Methoxyflurane in O_2	37.2±2.03	54.9±5.63	8	S
2.0% Halothane in O_2	39.2±2.68	44.1±6.40	6	NS
(Derived values comparing 20 mg% Thiopental Na with 10%)	20 mg% 59.1±5.48	10 mg% 59.8±8.87	7/6	NS

*S = significant, $P \leq 0.05$; NS = not significant, $P > 0.05$
+ Mean ± Sample Standard Deviation

DISCUSSION

The agents chosen for study have wide use in modern practice. Each has been examined in concentrations met in its clinical use. Although ordinarily two-thirds of thiopental in the bloodstream is bound to plasma proteins (Goldbaum and Smith 1954), the free arterial concentration during rapid induction may momentarily exceed 20 mg%. (Price et al. 1960) Comparison of 20 mg% to 10 mg% suggests that there may be a dose-response relationship at least in terms of the initial effects of the drug upon renal tubular function. Nitrous oxide was studied at the high end of the clinical range of concentration, while halothane and methoxyflurane were tested at more clinical levels. Methoxyflurane at 1.2% was about 5.2 MAC (minimal alveolar concentration)while halothane at 2.0% was around 2.3 MAC. Perhaps the more striking effects of methoxyflurane compared to control was due to this more than two-fold difference in clinical potency used. Nitrous oxide was at less than half its MAC, as no pressure facilities were available. (see Eger et al. 1965 for MAC values and a discussion of their significance)

Clearly, all agents studied, with the possible exception of halothane in the concentration tested, have significant effects on renal tubular transport processes, as measured by this method. It is noteworthy that the only in vivo study with renal vein PAH measured as a test of the method was with halothane

(Deutsch et al. 1966). Therefore, most prior studies of renal blood flow, measured by the traditional clearance techniques, must be considered as possibly not valid when used in anesthetized man or experimental animal. That the drugs should have such direct effects on processes at a cellular level should not be surprising, as other actions of a similar direct nature have been previously demonstrated. (see for example Andersen 1966 for a study of the effects of anesthetics upon cellular ion transport mechanisms, and Van Dyke et al. 1964 on the cellular metabolism of the drugs themselves) Perhaps some of the debate concerning the renal effects of methoxyflurane (Worth and Stephen 1965, also Crandell et al. 1966) may be related to the cellular depressant effects, if indeed there is true toxicity. From the introductory discussion presented before, it is clear that the ramifications may be widespread in medicine.

REFERENCES

Andersen NB: Effect of general anesthetics on sodium transport in the isolated toad bladder. Anesthesiology 27: 304-10, 1966

Berndt WO, Grote D: The accumulation of C^{14}-dinitrophenol by slices of rabbit kidnye cortes. J Pharmacol Exper Therap 164: 223-31, 1968

Beyer KH, Peters L, Woodward R, et al.:The enhancement of the physiological economy of penicillin in dogs by the similtaneous administration of para-amino-hippuric acid. J Pharmacol Exper Therap 82: 310, et seq., 1944

Bulger RE, Trump BF: A light and electron microscopic study of the mesonephric kidney of the English sole, Parophrys vetulus. Anat Rec 151: 445, et seq., 1965

Burg M, Grantham J, Abramow M, et al.: Preparation and study of fragments of single rabbit nephrons. Amer J Physiol 210: 1293-8, 1966

Burnett CH, Bloomberg EL, Shortz G, et al.: A comparison of the effects of ether and cyclopropane anesthesia on the renal function of man. J Pharmacol Exper Therap 96: 380-7, 1949

Cafruny EJ, Gussin RZ: Renal tubular excretion of mercurials in the aglomerular fish, Lophius americanus. J Pharmacol Exper Therap 155: 181-6, 1967

Chambers R, Kempton RT: Indications of function of the chick mesonephros in tissue culture with phenol red. J Cell

and Comp Physiol 3: 121-68, 1933

Coller FA, Rees VL, Campbell KN, et al.: Effects of ether and cyclopropane anesthesia upon the renal function in man. Ann Surg 118: 717-27, 1943

Craig FN, Visscher FE, Houck CR: Renal function in dogs under ether or cyclopropane anesthesia. Amer J Physiol 143: 108-18, 1945

Crandell, WB, Pappas SG, Macdonald A: Nephrotoxicity associated with methoxyflurane anesthesia. Anesthesiology 27: 591-607, 1966

Despopoulos A: Renal and hepatic transport of food dyes. J Pharmacol Exper Therap 163: 222-8, 1968

_____, Sonnenberg H: Congruence of excretory functions of liver and kidney: sulfonamides. Amer J Physiol 212: 1117-22, 1967

Deutsch S, Goldberg M, Stephen GW, et al.: Effects of halothane anesthesia on renal function in normal man. Anesthesiology 27: 793-804, 1966

_____, Pierce EC Jr, Vandam LD: Cyclopropane effects on renal function in normal man. Anesthesiology 28: 547-58, 1967

Eger EI II, Brandstater B, Saidman LJ, et al.: Equipotent alveolar concentrations of methoxyflurane, halothane, diethyl ether, fluroxene, cyclopropane, xenon and nitrous oxide in the dog. Anesthesiology 26: 771-7, 1965

Forster RP: Use of thin kidney slices and isolated renal tubules for direct study of cellular transport kinetics. Science 108: 65, 1948

_____, Renal transport mechanisms. Fed Proc 26: 1008-19, 1967

_____, Copenhaver JH Jr: Intracellular accumulation as an active process in a mammalian renal transport system in vitro: energy dependence and competitive phenomena. Amer J Physiol 186: 167-71, 1956

_____, Goldstein L: Relationship between renal succinoxidase activity and maximal transport rates of p-amino-hippurate (TmPAH) in various representative vertebrates. J Cell and Comp Physiol 58: 247-51, 1961

_____, Hong SK: In vitro transport of dyes by isolated renal tubules of the flounder as disclosed by direct visualization, intracellular accumulation and transcellular movement. J Cell and Comp Physiol 51: 259, et seq., 1958

_____, Sperber I, Taggart JV: Transport of phenolsulfonphthalein dyes in isolated tubules of the flounder and in kidney slices of the dogfish. Competitive phenomena. J Cell and Comp Physiol 44: 315-8, 1954

_____, Taggart JV: Use of isolated renal tubules for the examination of metabolic processes associated with active cellular transport. J Cell and Comp Physiol 36: 251-70, 1950

Freund JE: Mathematical Statistics. Englewood Cliffs, NJ, Prentice-Hall, Inc., 1962

Glauser KF, Selkurt EE: Effect of barbiturates on renal function in the dog. Amer J Physiol 169: 469-79, 1952

Goldbaum LR, Smith PK: The interaction of barbiturates with serum albumin and its possible relation to their disposition and pharmacological actions. J Pharmacol

Exper Therap 111: 197-209, 1954

Goldstein A, Aronow L, Kalman SM: Principles of Drug Action. New York, Hoeber Medical Div. of Harper and Row, 1968

Goodman LS, Gilman A, eds.: The Pharmacological Basis of Therapeutics. 3rd ed., New York, The Macmillan Co., 1965

Greene NM, ed.: Halothane. Philadelphia, F A Davis Co., 1968

Habif DV, Papper EM, Fitzpatrick HF, et al.: The renal and hepatic blood flow, glomerular filtration rate, and urinary output of electrolytes during cyclopropane, ether, and thiopental anesthesia, operation, and the immediate postoperative period. Surgery(St Louis) 30: 241-55, 1951

Hill LL, Daeschner CW Jr, Moyer JH: The influence of sedative agents upon renal hemodynamics. J Lab and Clin Med 52: 125-8, 1958

Hong SK, Forster RP: Run-out of chlorphenol red following luminal accumulation by isolated renal tubules of the flounder in vitro. J Cell and Comp Physiol 51: 241, et seq., 1958

_____, _____: Further observations on the separate steps involved in the active transport of chlorphenol red by isolated renal tubules of the flounder in vitro. J Cell and Comp Physiol 54: 237-42, 1959

Hook JB, Williamson HE: Influence of probenecid and alterations in acid-base balance of the saluretic activity of furosemide. J Pharmacol Exper Therap 149: 404-8, 1965

Jaffe OC: Phenol red transport in the pronephros and mesonephros of the developing frog (Rana pipiens). J Cell and Comp Physiol 44: 347-63, 19, 1954

Kennedy WF Jr, Sawyer TK, Gerbershagen HU, et al.: Systemic cardiovascular and renal hemodynamic alterations during peridural anesthesia in normal man. Anesthesiology 31: 414-21, 1969

Kinter WB: Chlorphenol red influx and efflux: microspectrophotometry of flounder kidney tubules. Amer J Physiol 211: 1152-64, 1966

May DG, Fujimoto JM, Inturrisi CE: The tubular transport and metabolism of morphine-N-methyl-C^{14} by the chicken kidney. J Pharmacol Exper Therap 157: 626-35, 1967

Mazze RI, Schwartz FD, Slocum HC, et al.: Renal function during anesthesia and surgery: I. The effects of halothane anesthesia. Anesthesiology 24: 279-84, 1963

Miles BE, DeWardener HE: Renal vasoconstriction produced by ether and cyclopropane anesthesia. J Physiol (London) 118: 140-4, 1952

_____, _____, Churchill-Davidson HC, et al.: The effect on the renal circulation of pentamethonium bromide during anesthesia. Clin Sci 11: 73-9, 1952

Miller JR, Stoelting VK, Rhamy RK: A comparison of the effects on renal tubular function of halothane-oxygen and halothane-nitrous oxide-oxygen anesthesia. Anesth and Analg 45: 41-3, 1966

_____, Townley N, Stoelting VK, et al.: Effect of halothane anesthesia on renal tubular function in man. Anesth and Analg 44: 236-9, 1965

Miller RH, Cabot H: The effect of anesthesia and operation on the kidney function, as shown by the phenolsulphonephthalein test. Arch Int Med 15: 369-91, 1915

Ngai SH, Papper EM: Metabolic Effects of Anesthesia. Springfield, Ill., Charles C Thomas Publishers, 1962

North BJ, Stephen CR: Hepatic and renal effects of methoxyflurane in surgical patients. Anesthesiology 26: 257-8, 1965

Pack TT, Wasserman K, Fishman AP: Some effects of inorganic ions on the active transport of phenol red by the isolated kidney tubules of the flounder. J Cell and Comp Physiol 40: 73-88, 1952

Papper EM: Renal function during general anesthesia and operation. JAMA 152: 1686-9, 1953

_____, Ngai SH: Kidney function during anesthesia. Ann Rev Med 7: 213-24, 1956

Papper S, Papper EM: The effects of preanesthetic, anesthetic, and postoperative drugs on renal function. Clin Pharmacol Ther 5: 205-15, 1964

Pitts RF: Physiology of the Kidney and Body Fluids. Chicago, Year Book Medical Publishers, 1963

Preston FW, Beal JM, eds.: Basic Surgical Physiology. Chicago, Year Book Medical Publishers, 1969

Price HL: Circulation During Anesthesia and Operation. Springfield, Ill., Charles C Thomas Publishers, 1967

_____, Kovnat PJ, Safer JN, et al.: The uptake of thiopental by body tissues and its relation to the duration of narcosis. Clin Pharmacol Ther 1: 16-22, 1960

Raventós J: The action of fluothane: a new volatile anaesthetic. Brit J Pharmacol 11: 394, et seq., 1956

Smith HW: From Fish to Philosopher. Summit, NJ, CIBA, 1959

Smith JM, et al.: Inhibition of the renal tubular transport and excretion of tetraethylammonium and N1-methyl-nicotinamide by non-nitrogenous onium compounds. J Pharmacol Exper Therap 158: 451-9, 1967

Sodeman WA, Sodeman WA Jr,eds.: Pathologic Physiology: Mechanisms of Disease. Philadelphia, W B Saunders Co., 4th ed., 1967

Sugiki S, Constant MA, Becker B: In vitro accumulation of chlorphenol red by rabbit ciliary body. J Cell and Comp Physiol 58: 181-3, 1961

Taggart JV: Mechanisms of renal tubular transport. Amer J Med 24: 774-84, 1958

Trump BF, Bulger RE: Studies of cellular injury in isolated flounder tubules: I. Correlation between morphology and function of control tubules and observations of autophagocytosis and mechanical cell damage. Lab Invest 16: 453-82, 1967

Van Dyke RA, Chenoweth MB, Van Poznak A: Metabolism of volatile anesthetics: I. Conversion in vivo of several

anesthetics to $^{14}CO_2$ and chloride. Biochem Pharmac 13: 1239-47, 1964

Weast RC, Selby SM, Hodgman CD, eds.: Handbook of Chemistry and Physics. Cleveland, O., Chemical Rubber Co., 45th ed., 1964

Weiner IM, Mudge GH: Renal tubular mechanisms for excretion of organic acids and bases. Amer J Med 36: 743-62, 1964

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